Fabrication and Characterization of a Polymer Based Three Compartmental Scaffold for Fibrocartilage Regeneration

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Certificate

This is to certify that the report entitled, "**Fabrication and Characterization of a Polymer Based Three Compartmental Scaffold for Fibrocartilage Regeneration**", submitted by **Mrs. RASHMI REKHA BEHERA**, Roll No.:**111BT0015**, B-Tech-8th semester, Department of Biotechnology & Medical Engineering, National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by her under my supervision and guidance.

To the best of my knowledge, the matter embodied in the report has not been submitted to any other University / Institute for the award of any Degree or Diploma.

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I am sincerely thankful to **Prof. Kunal Pal,** Dept. of Biotechnology and Medical Engineering, NIT, Rourkela, for providing the necessary facilities for this work.

Special thanks to **Mr. Vinay Kumar and Mr. Rakesh** for being with me during my ups and downs of my project.A friend in need is a friend indeed.If this is true then all labmates are my true friends who pointed out my mistakes while doing project and helped me to find the solutions.

Finally, I would like to express my thanks to my parents for their blessings, support and constant encouragement and very special thanks to God for showering the blessings on me.

Date: 11-05-2015 **RASHMI REKHA BEHERA**

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ABSTRACT

Tissue engineering applies the principles of biology and engineering to the development of viable substitutes that restore, maintain or improve the function of human tissues. It depends upon a temporary scaffold that is an architecture that functions to mechanically support the cells and regulate the functions of cells in a manner analogous to extracellular matrix of mammalian tissue. It also assist in 3D tissue formation or organogenesis. The scaffolds must have the capability to incorporate mammalian tissue and assist in their growth, migration and differentiation which will help in tissue regeneration. Due to its unique properties, it is used in implantation. The most common injuries include the damage of cartilages, tendons and ligaments. So globally musculoskeletal tissue engineering is given undue importance. During ligament injury the injured ligaments are not successfully replaced by ligament-alone grafts. Thus there is a need for the construction of a composite scaffold to allow stem cell to differentiate into fibrocartilage that bridges of Bone-Ligament interface. The current project has focused on the fabrication of a silk-based knitted scaffold for fibrocartilage regeneration. Bio-polymers such as silk solution and chitosan were used for surface modification of the knitted silk scaffold, to ensure differentiation of the MSCs into fibrocartilage lineages. Lyophilization process was used for coating of polymers over the knitted silk scaffold. Characterization of the polymer coated knitted silk scaffold was done using XRD, FE-SEM, EDX, FTIR. Water absorption capacity of the knitted silk scaffold was also studied. It was concluded that the multi-compartment silk based scaffold developed in house has the novelty for tissue engineering the bridge component between bone and ligament i.e. fibrocartilaginous enthesis.

KEYWORDS*:* Extracellular matrix, knitted scaffold, Fibrocartilage regeneration, Organogenesis, Cartilage, Tendon, Ligament, Musculoskeletal tissue engineering, Fibrocartilage, Bone-Ligament interface, Bio-polymers, Lyophilization

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CHAPTER-1
INTRODUCTION

1.1 INTRODUCTION

Recently scaffold was developed as a new process to create a hard tissue (bone)-soft tissue (cartilage or ligament) bilayered. Musculoskeletal disorders have been converted into one of the major health concerns because of sports-related injuries and aging population. Bone repair commonly includes the utilization of allografts or autografts, ligament repair mostly uses patellar tendon, whereas cartilage repair requires use of cartilaginous grafts or joint resurfacing. Using prosthetics, total joint replacements are done in most cases of musculoskeletal disorder. This procedure cannot generally be functional because of deficiency in the supply of donor tissue. There was a risk of immunorejection and disease transfer while obtaining tissue from other source.[1]

By applying the principles of engineering and biology, Tissue engineering may be a way to avoid the limitations of existing therapies in solving the problems associated with partial or whole organ transplantation.

One of the methodology is to utilize three dimensional porous, biodegradable polymeric scaffolds. As the scaffold degrades devices have been used it allows support for the in growth of new tissue. [2]

Another methodology is to culture the cells on a preformed three-dimensional scaffold and transplant the cell-polymer construct into the patient.[3]

In the field of nanotechnology Electrospinning is considered to be one of the most promising processes because of its simplicity, ease, low cost, high productivity, high efficiency, reproducibility and its potential to be scaled up to the industrial scale . A high voltage electric field is involved in this process to draw very thin fibres from a polymeric fluid stream which was delivered through a millimetre-scale nozzle.[4] Electrospinning method relies upon various parameter such as polymer solution parameters (e.g., viscosity, surface tension, dielectric constant, concentration and conductivity) and processing parameters (e.g., electric field strength, solution flow rate, needle diameter and distance between needle tip and ground collector).[5]

Nano fibre is characterized as the fibre having at least one dimension in nanometre range. Nano fibre is utilized for an extensive variety of medical applications for drug delivery systems, scaffold formation, wound healing and widely used in tissue engineering, skeletal tissue, bone tissue, cartilage tissue, neural tissue, ligament tissue, etc.[6]

CHAPTER –2
LITERATURE REVIEW

2.1 LITERATURE REVIEW:

2.1.1 TYPES OF CARTILAGE

2.1.1.1 HYALINE CARTILAGE

Hyaline cartilage forms the articular surfaces of long bones, the rib tips, the rings of the trachea and parts of the skull in adults. It is the most widespread cartilage type and its name refers to its glassy appearance. It is predominately collagen.[7] In the embryo, bones form first as hyaline cartilage before ossifying as development progresses. With the help of a fibrous membrane named as perichondrium hyaline cartilage is externally covered, except at the articular ends of bones and also where it is found directly under the skin like ears and nose. The vessels are present in the membrane, which provide nutrition to the cartilage. [8]

When observed under the microscope , rounded or blunty angular form type cells can be found in groups of two or more in a granular or almost homogeneous matrix .These cells have generally straight outlines where they are in contact with each other, whereas the rest of their circumferences are rounded. Translucent protoplasm is present which sometimes contain fine interlacing filaments and minute granules. [9]

2.1.1.2 FIBROCARTILAGE

Fibrous cartilage is composed of lots of collagen fibres (type I and type II) whereas a mixture of white fibrous tissue and cartilaginous tissue is present in white fibrocartilage in various proportions. Its flexibility and toughness are the primary and elasticity is the secondary advantage to these constituents. It is the only type of cartilage that contains type I collagen in addition to the normal type II. joint Pubic symphysis, the annulus fibrosus of intervertebral discs, menisci, and the TMJ contain fibrocartilage. Relaxin loosens the pubic symphysis to aid in delivery during labor but this can cause some problems. [10]

2.1.1.3 ELASTIC CARTILAGE

The most important constituents of elastic or yellow cartilage are elastic fibre and collagen fibre. Elastin is the principal protein. Histologically it is similar to hyaline cartilage but the solid matrix is filled with many yellow elastic fibres. The bundles made from these fibres appear dark under a microscope. It is able to withstand repeated bending because they give great flexibility to this elastic cartilage. The chondrocytes are present in between the fibres. It is generally found in the pinnae (the external ear flaps of many mammals including humans) and in the epiglottis (part of the larynx). With verhoeff stain the elastin fibres turn dark purple/black.[11]

2.1.2 FIBROCARTILAGE

The cartilage which is very rich in type I collagen is known as Fibrocartilage and mostly found in areas such as the meniscus of the knee, the pubic symphysis and the vertebral discs. Due to its strength and durability, it appears in areas where these traits are needed. Injury

takes place in the form of tearing. To repair a tear of fibrocartilage or to stabilize the area where the tear occurs, surgery is mostly done so that healing can take place.[12]

The characterization of this form of cartilage is done by the clearly visible-bundles of tough collagen under a microscope. The bundles are amalgamated with clefts which contain cartilage cells, and the cartilage includes a mixture of types I and II collagen, along with other components of cartilage. The bundles of collagen are responsible for making fibrocartilage so tough, while individual strands can undergo breaking, others will retain their strength and act like a supportive structure to keep the cartilage from being compromised. Fibrocartilage is needed to provide support between the vertebrae along the spine also it offers some protection to the spinal cord. Jointed spine is the one which allows organisms to bend, but vulnerability is also created at each joint due to it. Fibrocartilage provides a surface for articulation so that the vertebrae can move smoothly when someone bends or twists the spine also supports the spine and the joints of the spine, absorbs shocks etc. [13]

The cartilaginous joint which comprises part of the pubic bone in the public symphysis, fibrocartilage holds the joint together, but during pregnancy it can also soften and pull apart to allow the expanding uterus to fit. It pulls apart even further during the time of labour and delivery, so that the baby can get out, before firming back up again after pregnancy to stabilize the pelvis. This softening depends upon the release of a hormone named as relaxin.[14]

In joints such as the knee, fibrocartilage is not only involved in the articulation of the joint but also helps in protection of the joint. This type of cartilage has a lot of use and can be subjected to heavy impacts as it is less prone to tearing and separation than other forms of cartilage, so most of these are appears in several other joints. Pain, soreness in the joint and stiffness are caused due to the problems with the cartilage in a joint and eventual lead to damage of the bone as the padding of cartilage is worn away.[15]

2.1.3 ROLES OF FIBROCARTILAGE

Fibrocartilage provides high tensile strength and support. It is present in areas most subject to frequent stress like intervertebral discs, the symphysis pubis and the attachments of certain tendons and ligaments. It is important in providing restricted mobility and attaching bone to bone. It's main function is to reduce friction between the joints and provides rigidity to the surrounding structures. It is the strongest among the three types of cartilage.[16]

2.1.4 SCAFFOLDS:

For the cells growth and production of cartilage tissue and extracellular matrix, scaffold provide appropriate environment. To avoid differentiation of their phenotype chondrocytes require 3D culture. Furthermore, when chondrocytes are relocated into a three-dimensional (3D) environment the process of differentiation can be reversed. From a diverse range of materials including natural or synthetic materials or a hybrid of both, the scaffolds can be constructed. [17]Hydrogels, sponges and fibrous mesh are different forms to which the scaffold can be designed. Hydrogels support the transportation of cells and bioactive agents and can suspend cells in a three dimensional environment. The defects of any size and shape can be filled by injection of them. However they have inferior mechanical properties

compared with other forms of scaffolds. Cell adhesion is done by Sponges which are porous scaffolds. Cell adhesion, migration and deposition are affected by variation in pore size. Depending on fibre diameter and direction meshes can also be made to variable porosities. They exhibit greater mechanical strength but irregular filling into the mesh itself may compromise the quality of the graft and affect tissue integration. To mimic the native cartilage material and 3D environment, 3D constructs of woven fibres and electrospinning have been used.[18]

2.1.5 BIODEGRADABLE POLYMER

Polymers have functional groups which links monomers to one another and have backbone which is responsible for unstable links are known as biodegradable polymers. These polymers after metabolisation breaks down into biologically acceptable molecules and removed from the body via normal metabolic pathways. Based on biodegradability polymers are classified as 1. Biodegradable polymers eg: collagen, chitosan, silk etc. 2. Non biodegradable polymers eg: poly vinyl chloride, polyethylene etc.

The polymers which are going to be used in Tissue engineering should be Biodegradable means the rate of scaffold degradation should be equal to the rate of natural tissue regeneration.[19] Biodegradable polymers which refers to the rate of natural tissue regeneration should be equal to the rate of scaffold degradation are mainly used in Tissue engineering purposes.

2.1.5.1 CHITOSAN

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). With the alkali sodium hydroxide, it is made by treating shrimp and other crustacean shells. [20]

In chitosan the amino group has a pKa value of ~6.5, which leads to a protonation in acidic to neutral solution with a charge density dependent on pH and the %DA-value. Due to this chitosan is known to be water soluble and a bioadhesive which readily binds to negatively charged surfaces such as mucosal membranes. The transportation of polar drugs across epithelial surfaces are enhanced by chitosan and is biocompatible and biodegradable.[21]

2.1.5.2 SILK

Each fibre of Bombyx mori silk consists of its silk coating (sericin protein) & an inner core (fibroin protein), are usually 10-20 micron in thickness. We can get nanofibres from the fibroin, which are present as parallel fibrils. Because of the toxic nature sericin protein is removed. The properties of silk are quite unique due to the unique amino acid composition of silk which is translated into a specific primary structure.[22]

2.1.6 FABRICATION TECHNIQUES:

Tissue engineering scaffolds plays an important role for building functional tissues and organs which should be sorted into 3D architecture inside body and need to be fabricated utilizing different strategy for facilitating uniform cell distribution and managing their proliferation & differentiation.[23]

2.1.6.1 SOLVENT CASTING:

Solvent casting is a process by which thermoplastic polymer samples can be formed by dipping a mould into a solution of the polymer and drawing off the solvent to leave a polymer film adhering to the mould. This method of scaffold preparation is very simple and cost effective. The solvent can denature protein because of toxicity is the main disadvantage of this method. By vacuum process, this problem can be overcome but it is very much time consuming.[24]

2.1.6.2 PARTICULATE LEACHING:

Particulate leaching is a technique in which the pores can be created inside the mould using porogens like wax, salt or sugar. Here, by changing the porogen size, the size of pores can be modified. A polymer solution is casted into the mould filled with porogen and after evaporation of the solvent, leaching out of the porogen crystals is done using water which forms desired pores in the scaffold.[24]

2.1.6.3 GAS FOAMING:

High temperature and organic solvents are not involved in this technique. For fabricating the scaffold with high porosity, it uses CO_2 gas with high pressure which depends upon the amount of gas dissolved in the polymer. For constructing 3D porous scaffold it depends upon the porogen size after completion of foaming process. The mixture of porogen and polymer are exposed to high pressure, until the completion of saturation with CO_2. Incorporation of a particulate leaching method on gas forming help to get opened pores on the surface of a scaffold [25]

2.1.6.4 PHASE SEPARATION:

Phase separation is division of polymer solutions into two phases one with low polymer concentration and other with high polymer concentration. After phase separation the concentrated phase solidifies , and forms the scaffold. Three-dimensional network is formed with a porous structure. There is enhancement of cell adhesion, migration, proliferation and differentiation function due to high surface area-to-volume ratio of scafflod. It can give better result combined with particulate leaching and rapid prototyping.[26]

2.1.6.5 ELECTROSPINNING:

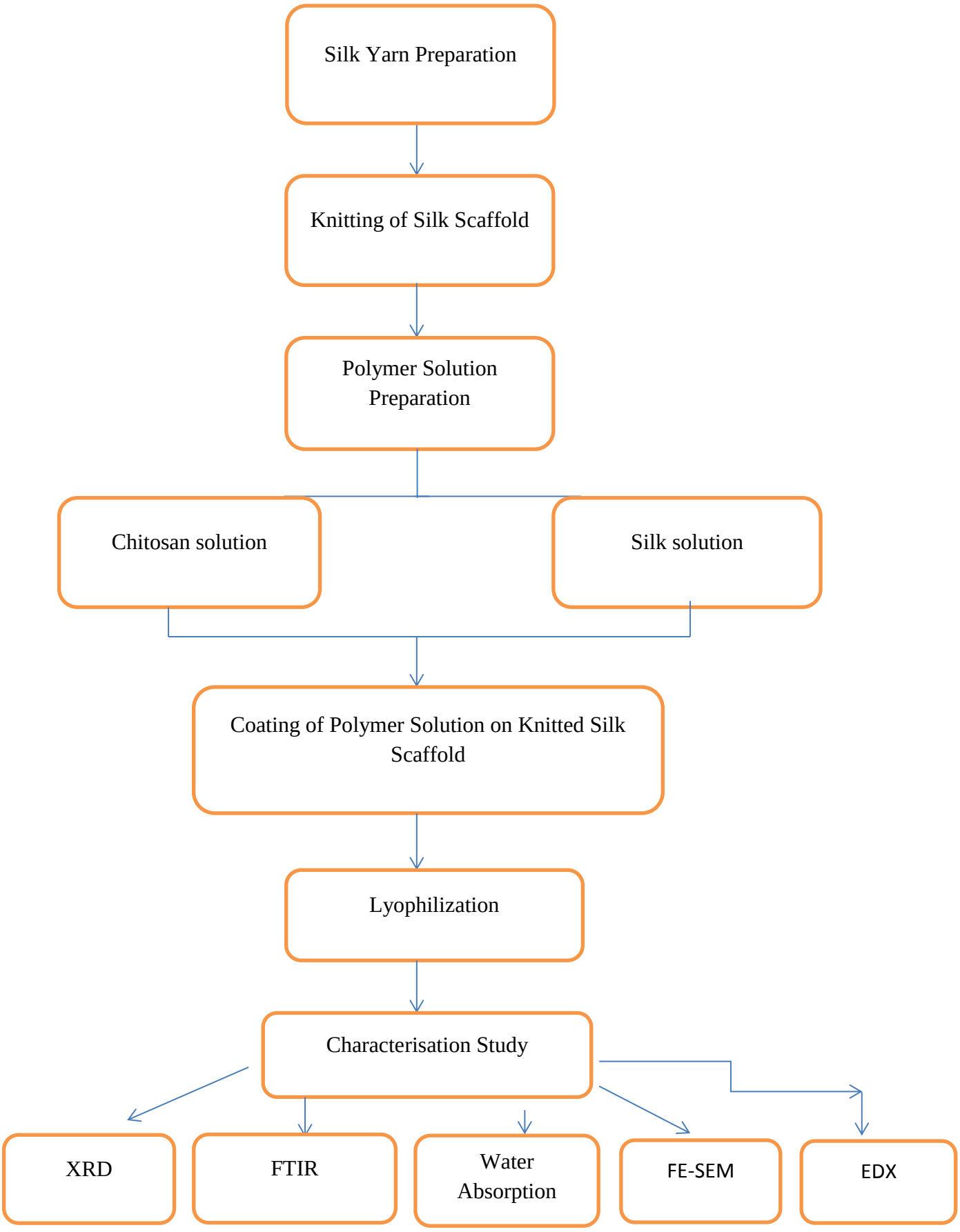
Nanofibres are produced from the polymeric solutions. The principle of operation is that high voltage (10kv to 50kv) is applied between two electrodes.Thus there are opposite charge polarity. The concentration of polymer solution, voltage supply and distance between the collector plate and the solution container determines the thickness of fibre. [27]

It is a method of fabrication of nanofibres using electrostatic field from the polymeric solutions. Polymer solution is forced through a capillary due to intervention of high voltage supply (10kv to 50kv) between two electrodes having opposite charge polarity resulting in formation of nanofibres. The thickness of fibre produced depends on the concentration of polymer solution, voltage supply and distance between the collector plate and the solution container. Both random and oriented fibres can be fabricated depending upon the collector.[28]

2.1.6.6 FIBRE MESH:

This technique involves fibre which may be made into 3D pattern having different porosity. To achieve this firstly there is a deposition of polymer solution over a nonwoven mesh of different polymer and then evaporation. Large surface area for cellular attachment and quick diffusion of nutrients are provided by evaporation. But its structural stability is very poor. [29]

CHAPTER – 3
WORK PLAN


Silk Yarn Preparation
Knitting of Silk Scaffold
Polymer Solution Preparation
Chitosan solution
Silk solution
Coating of Polymer Solution on Knitted Silk Scaffold
Lyophilization
Characterisation Study
XRD
FTIR
Water Absorption
FE-SEM
EDX

CHAPTER – 4
MATERIALS AND METHODS

4.1 FABRICATION OF SCAFFOLD

4.1.1 PREPARATION OF SILK YARN

From Raw Tasar Silk Depot the silk fibres were procured, Chiabasa, Jharkhand, India. The silk strand having 12 fibres was used out of the many types of silk fibres that were procured. Stretching of the fibroin was done and woven around to form a 12-threaded yarn. For the formation of a cluster thread of roughly 2mm thickness this yarn was then braided. To prepare the knitted-silk base for the scaffold this silk thread was used further.

Figure 1(a): Structure of raw silk

Figure 1(b): Structure of coiled silk

4.1.2 KNITTING OF SILK

For making yarns the woven silk fibroin threads were used which were knitted using Brother Knitting Machine (Model no. KH830) using 4 alternative needles to obtain a patterned mesh of dimension 2cmX4cm with present pore patterns. Four alternate needles were pushed to

'Out' position to carry out the knitting process. The K-carriage was loaded with the yarn threads and pulled towards the right end. The four needles were shifted to 'Working' position. To provide uniform force towards the ground the 'Claw' weights were used during knitting process. The knitted silk was then directed downwards. To operate the knitting process the K-carriage was alternatively slid left and right over the four needles. After completion of knitting process, the remaining yarn was cut about 10cm from the main knitted silk and the K-carriage was moved away from the knitted silk. Then on to a rectangular glass slide of dimension 3cmX8cm the knitted mesh was tied such that the mesh is exposed on one side of the glass slide.

Figure 2 (a) : Knitting Machine (Brother Knitting Machine Model no. KH830)

Figure 2 (b) : Knitted silk (Mesh form)

Figure 2: (c) Knitted silk scaffold tied on glass slide

4.1.3 MORPHOLOGICAL CHARACTERIZATION OF KNITTED SILK

Field emission scanning electron microscope using platinum coating was used to determine the morphological characteristic of the knitted silk. To load on the sample holder of the device, the knitted silk was cut into small square pieces of suitable dimensions. The fibres were subjected to sputter coating of platinum for 8 minutes in JEOL JFC-1600 Autofine coater to visualize under the microscope. Under an electric field of 15KV the knitted silk was observed and was visualized at 30x, 50x and 150x magnification.

4.1.4 PREPARATION OF PHOSPHATE BUFFERED SALINE

For preparation of experimental solutions and in vitro studies one of the most important buffers used is Phosphate Buffered Saline (PBS), because of its constituent and parametric resemblance to the body fluids. Preparation of 1L of 1X PBS solution was done. 8g of NaCl was mixed thoroughly followed by 0.2g of KCl. By the addition of 1.44g of Na_2HPO_4 and then KH_2PO_4 this process was done. By using 1N NaOH and 1N HCl the pH of the solution was adjusted until the value was set at 7 at 25ºC.

4.2 POLYMER COATING OF KNITTED-SILK SCAFFOLD

4.2.1 PREPARATION OF SILK SOLUTION

2% Silk solution in 98% formic acid

- For 100ml of solution
  2gm silk + 100ml formic acid

- Hot plate stirrer (temp. 45-50^{0}C , time 9-10 hrs)

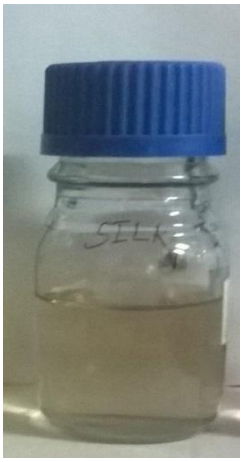


Figure 3: Silk solution

4.2.2 PREPARATION OF CHITOSAN SOLUTION

2% Chitosan solution

- For 100ml solution
  2gm chitosan + 100ml water +20 ml of acetic acid

- Hot plate stirrer (without temp. , time 4 hrs)

Figure 4: Chitosan solution

STEPS:

- The silk solution was filtered with the help of a filter paper.

- After filtration the silk solution was measured and same amount of chitosan solution was added to it.

- 2ml of glutaraldehyde and 2 ml of formic acid was added to it.

4.2.3 PREPARATION OF SILK/CHITOSAN COATED KNITTED SILK SCAFFOLD

The solutions of the two polymers were mixed together such that the resultant solution contained equal concentration of both silk and chitosan. Five different knitted silk scaffolds each of dimension 1cm×1cm were taken in five different petridish of 35mm. The polymer solution (chitosan/silk) was poured on four petridish except the one which was normal. The samples were immersed with in the polymer solution for 3hours (for 1st sample), 6 hours (for 2nd sample), 9 hours (for 3rd sample) and 12 hours (for 4th sample) respectively. After completion of time period all the sample were taken and kept at room temperature. Then the samples were proceed for lyophilisation.

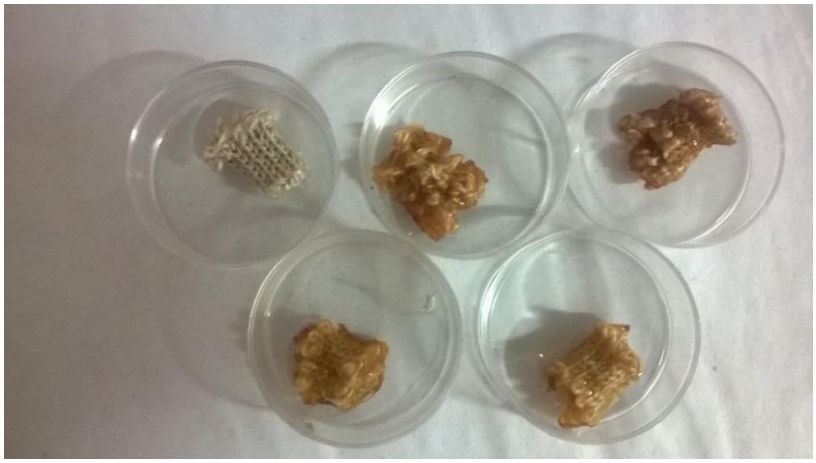


Figure 5: Silk/chitosan coated knitted silk scaffold

4.2.4 LYOPHILIZATION/ FREEZE DRYING PROCESS

To preserve a perishable material or make the material more convenient for transport a dehydration process is typically used known as lyophilization. Freeze-drying works by solidifying the material and then lowering the surrounding pressure to allow the frozen water in the material to sublimate straightforwardly from the solid phase to the gas phase. The samples were kept at -20℃ for 24 hours for freezing process to take place. They were put inside the lyophilizer to continue lyophilisation process. The samples were kept for 24 hours inside the lyophilizer to allow the frozen water in the material to sublimate directly from the solid phase to the gas phase.

Figure 6: Lyophilised silk/chitosan coated knitted silk scaffold at different time period

4.3 CHARACTERIZATION STUDIES

4.3.1 WATER ABSORPTION STUDIES

Studies of water absorption properties are important for every kind of scaffold. At pH 7 PBS solution was made. Introductory dry weights of all three scaffolds were noted and afterward they were drenched in PBS at 37ºC (denoted as W0). The weights of scaffolds were checked each 60 minutes and were drenched back in fresh PBS arrangement under the same conditions. The recently obtained weights were noted until there was no intermittent change in the weight of the sample (final weight meant as Wn where "n" the last drenching step). This was taken as the highest absorption capacity of the scaffold. The percentage of water absorption of the scaffold was calculated using the following formula:

$$\%\text{Absorption} = (Wn - W_0)/W_0 \times 100$$

The affinity of the scaffold material with water molecules and the size of the scaffold determine the absorption capacity of water.

4.3.2 FIELD EMISSION SCANNING ELECTRON MICROSCOPY

(FE-SEM)

Field emission scanning electron microscope was used to study the topology of the silk/chitosan coated knitted scaffold. The sample of knitted scaffolds were cut in proper dimensions and sputter coated under vacuum with particulate platinum for a duration of 8 minutes. The scaffolds were then moved to high vacuum chamber of the FE-SEM and were visually observed for their characteristics at magnifications of 30X, 50X and 150X.

4.3.3 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Silk/chitosan coated samples were subjected to Fourier Transform Infrared Spectroscopy. It was completed utilizing Alpha Bruker FTIR Spectroscopy, Shimadzu Corp. (Japan). The samples were cut into very fine pieces and pressed into pellets and set on the sample holder. The infrared peaks were obtained for the Scaffold coated with different concentrations of silk/chitosan solution.

4.3.4 X-RAY DIFFRACTION (XRD)

Studies of X-Ray Diffraction were performed for silk/chitosan coated knitted silk scaffolds with different concentrations of coatings . XRD was completed utilizing Rigaku Ultima IV X-Ray Diffractometer at an observational range of 10º-60º with a step size of 0.04º and at a rate of 3º/min. Under 60kV an energy of 2kW was supplied to A-41-Copper electrodes emitting beams through a window of 10mm width.

4.3.5 ENERGY-DISPERSIVE X-RAY SPECTROSCOPY (EDX)

For the analysis of element or chemical characterization of a sample, an analytical technique used is Energy-dispersive X-ray spectroscopy. Here a source of a sample is in interaction with excitation of X-ray. It's basic principle is that X-ray emission spectrum shows unique set of peaks which is due to unique atomic structure in each element. The emission of characteristic X-rays from a specimen is stimulated by a high-energy beam of charged particles such as electrons or protons (see PIXE), or a beam of X-rays which acts on sample.

CHAPTER - 5
RESULTS AND DISCUSSION

5.1 MORPHOLOGY OF SCAFFOLD

5.1.1 MORPHOLOGY OF KNITTED SCAFFOLD

The knitting of silk was done with the help of knitting machine (Brother Knitting Machine, China).For the morphological analysis of the knitted silk scaffold, which was prepared by knitting 12-yarn silk fibres, was visualized under FE-SEM at 30x. The figure (7) describes coiling of the silk fibres around each other forming a mesh like structure which are interconnected.

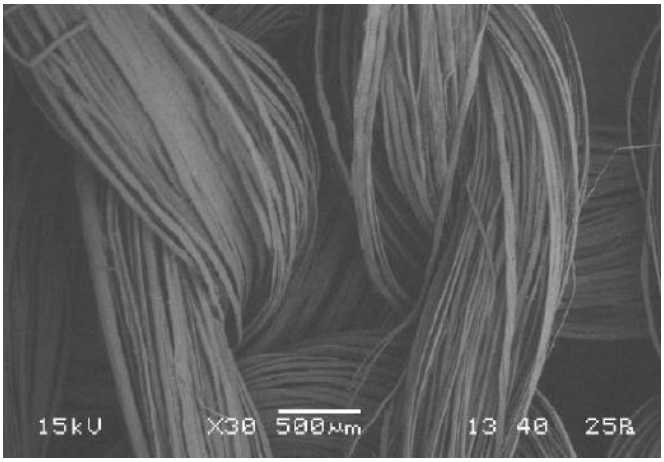


Figure 7: Morphological visualization of knitted silk based scaffold under FE-SEM

5.1.2 MORPHOLOGY OF SILK/CHITOSAN COATED SCAFFOLD

Silk/chitosan solution was prepared by adding equal amount and concentration of both the mixture**.** The knitted silk scaffolds were immersed in the solution and proceed for lyophilisation process. In this process 1st freezing was done and then by reducing the surrounding pressure sublimation of the frozen water takes place in the material from solid phase to gas phase**.** The polymer solution was coated on the knitted silk scaffold and different samples were prepared at different time period. There was a good coating of silk/chitosan solution on the knitted silk scaffold which was subjected for 12 hours.

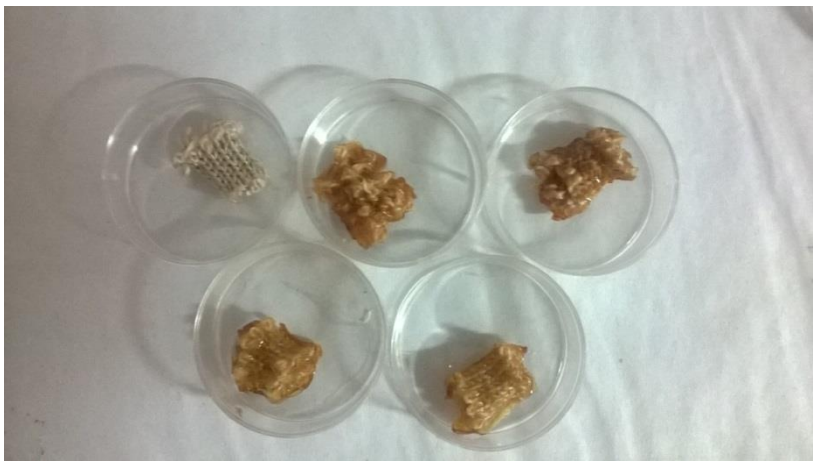


Figure 8: Knitted silk scaffold after coating with silk/chitosan solution

5.1.2.1 SEM ANALYSIS OF SILK/CHITOSAN COATING

SEM analysis gives the information about the presence of silk/chitosan coating on the surface of the knitted silk scaffold. With increase in time period the coating of polymer solution becomes thickened. FE-SEM analysis revealed the topology of the silk/chitosan coated knitted scaffold

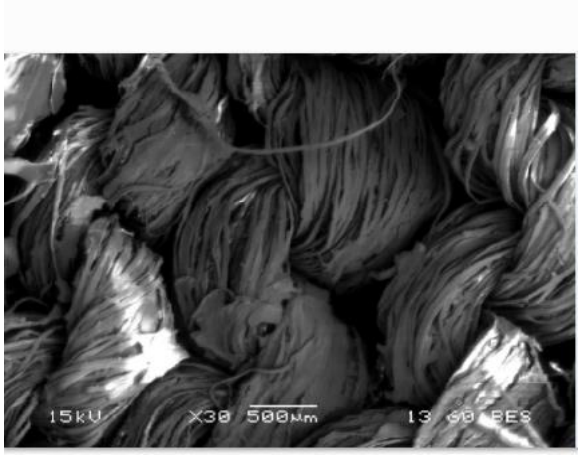


Figure 9(a): SEM micrograph of silk/chitosan coated knitted silk based scaffold in 30X

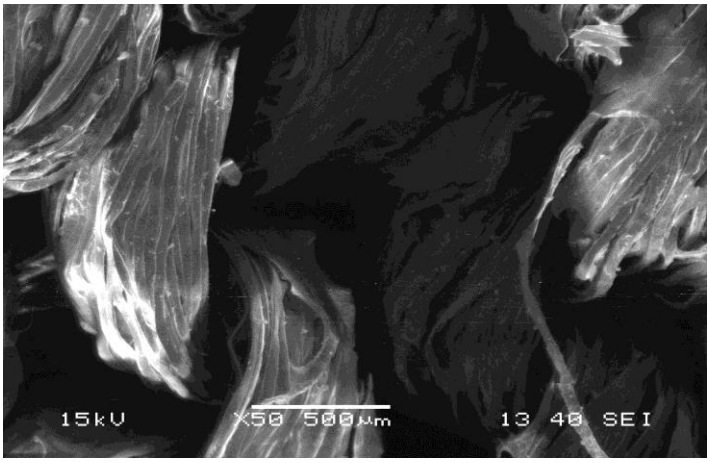


Figure 9(b): SEM micrograph of silk/chitosan coated knitted silk based scaffold in 50X

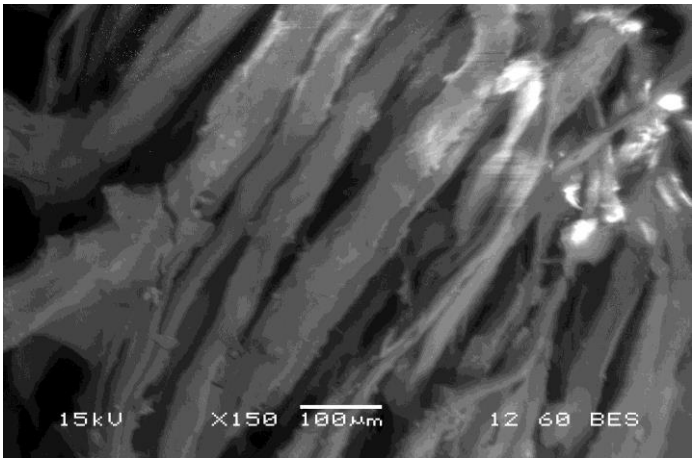


Figure 9(c): SEM micrograph of silk/chitosan coated knitted silk based scaffold in 150X magnifications

5.1.2.2 XRD ANALYSIS OF SILK/CHITOSAN COATING

The XRD peak of silk/chitosan coated knitted silk scaffold shows a broad Peak around 2θ=20.80 for chitosan and another peak around 2θ=31.3 for silk (Figure 10). XRD analysis was performed to determine the crystallinity of the silk/chitosan coating formed on the knitted silk scaffold.

Peak List

General information

Analysis date	2015/04/24 17:01:58		
Sample name	silkchitosan	Measurement date	2015/04/24 15:56:06
File name	silkchitosan.raw	Operator	pky8
Comment			

Peak list

No.	2-theta(deg)	d(ang.)	Height(cps)	FWHM(deg)	Int. I(cps deg)	Int. W(deg)	Asym. factor
1	17.18(10)	5.16(3)	1796(47)	2.9(8)	5527(1689)	3.1(10)	0.6(3)
2	20.80(11)	4.27(2)	3970(70)	2.7(3)	11297(2549)	2.8(7)	1.1(2)
3	24.28(10)	3.663(15)	2880(60)	3.9(6)	12084(2129)	4.2(8)	0.9(3)
4	31.3(5)	2.86(4)	450(24)	8(2)	3604(1141)	8(3)	3(4)
5	44.91(10)	2.017(4)	448(24)	3.4(3)	1900(179)	4.2(6)	2.2(12)

Measurement profile

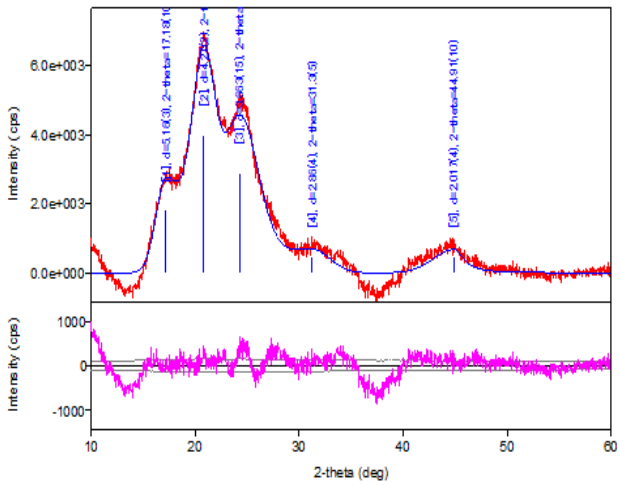


Figure 10: XRD of silk/chitosan coated knitted silk based scaffold

5.1.2.3 FTIR ANALYSIS OF SILK/CHITOSAN COATING

FTIR analysis was carried out to determine the side chains present in the sample and characteristics of bond. The major peak at around 1250 cm^{-1} is indicative of the presence of amide III linkage of silk fibroin. The peaks around 2922 cm^{-1} and 1380 cm^{-1} are indicative of amine group of chitosan. Peak around 3444 cm^{-1} indicative of OH^- group of chitosan. (Figure 11).

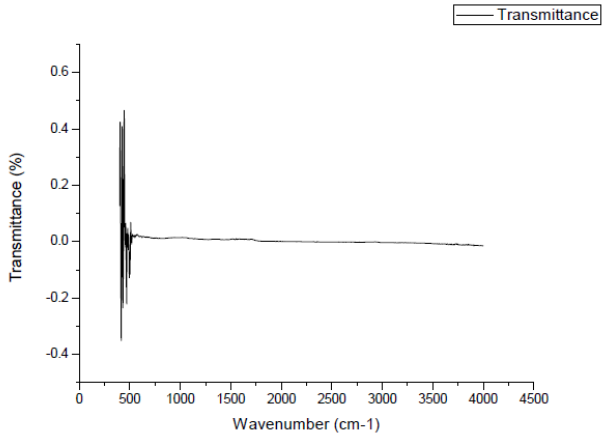


Figure 11 (a): FTIR analysis of 1st sample (3 hrs)

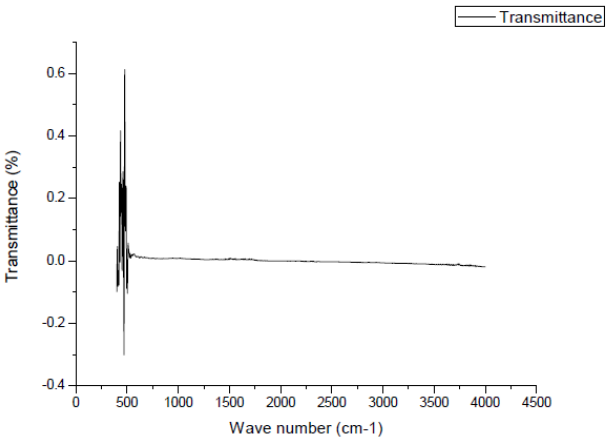


Figure 11 (b): FTIR analysis of 2nd sample (6 hrs)

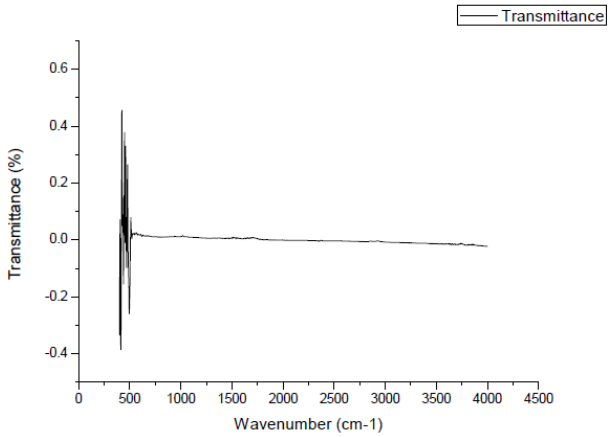


Figure 11 (c): FTIR analysis of 3rd sample (9 hrs)

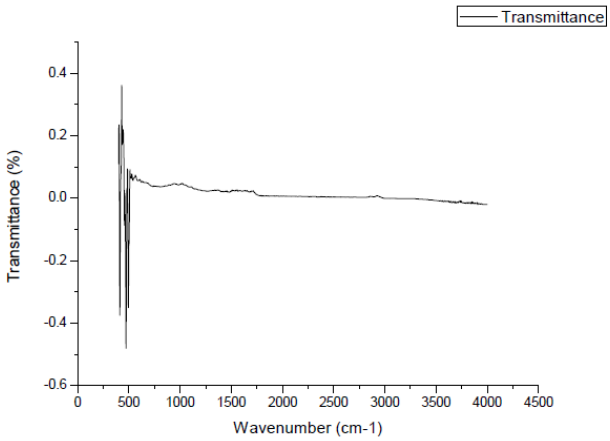
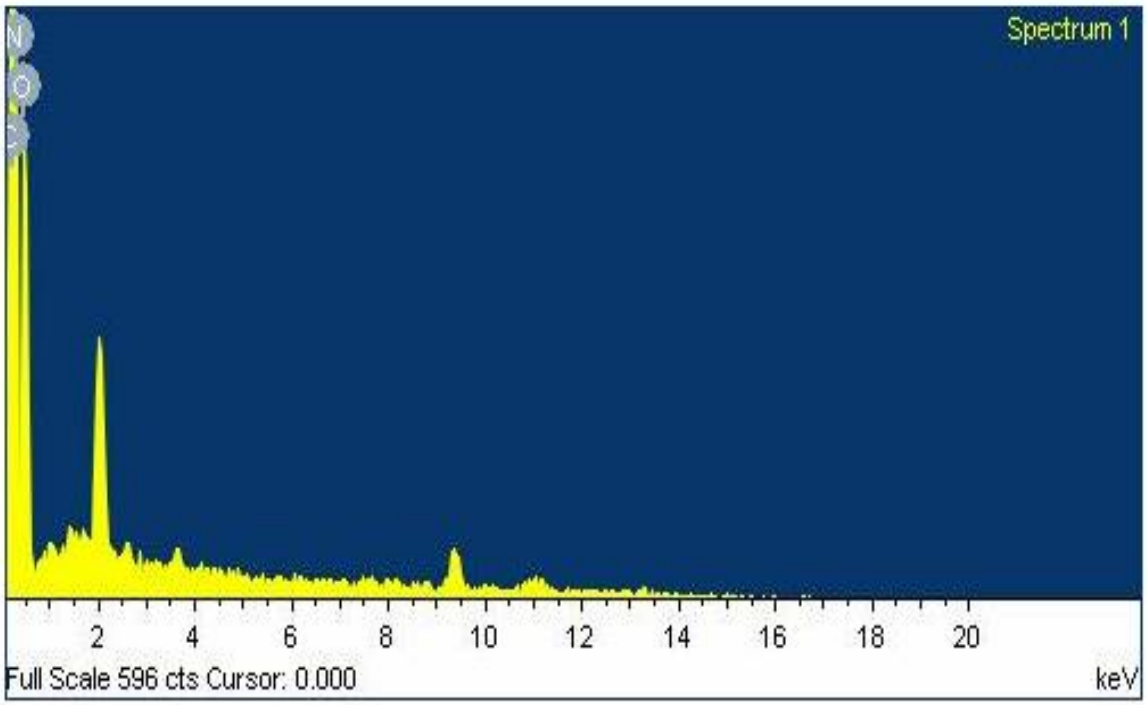


Figure 11 (d): FTIR analysis of 4th sample (12 hrs)

5.1.2.4 EDX ANALYSIS OF SILK/CHITOSAN COATING

EDX is used for the elemental analysis of the silk/chitosan coated knitted silk scaffold. It gives peaks at different wavelength showing different elements in the sample. Different peaks were observed for elements like carbon, oxygen, nitrogen at different wavelength for the silk/chitosan coated knitted silk scaffold.



Element	Weight%	Atomic%
C K	56.72	63.54
N K	0.52	0.50
O K	42.76	35.96
Totals	100.00	

Fig 12: EDX analysis of silk/chitosan coated knitted silk scaffold

5.2 WATER ABSORPTION STUDIES

To find out the water uptake capability of the knitted silk scaffold coated with various polymeric materials, water absorption study was done. At 37ºC immersion of scaffolds were done in PBS solution. Observation of the changed weight of the scaffold was done for an interval of 120 minutes assuring sure that there was no significant change in the wet weight of scaffold. The water uptake capability of the scaffold was calculated as the difference between the final weight and the initial weight.

5.2.1 WATER ABSORPTION STUDY FOR SILK/CHITOSAN

From the study we got the result that, the brittleness of the sample increases with increase in concentration of silk/chitosan when dipped in water and started getting separated from the knitted silk construct. The water uptake rate and concentration of silk/chitosan was directly related, the more is the concentration the more is the water absorption of the silk/chitosan coated knitted silk mesh. The saturation point at which no significant absorption takes place was observed around 360minutes onwards. For the production of a novel and ideal scaffold, the lowest concentration of silk/chitosan coated knitted silk scaffold was preferred (Figure 13, 14).

Water absorption studies

Time(min)	silk/chitosan coated knitted silk scaffold Weight (gms)				
	N	S1	S2	S3	S4
0	0.128	0.158	0.178	0.195	0.206
120	0.314	0.326	0.341	0.444	0.681
240	0.205	0.317	0.238	0.303	0.589
360	0.306	0.330	0.312	0.403	0.689
480	0.221	0.310	0.293	0.355	0.651

Fig 13: Water absorption study (table) of silk/chitosan coated knitted silk scaffold

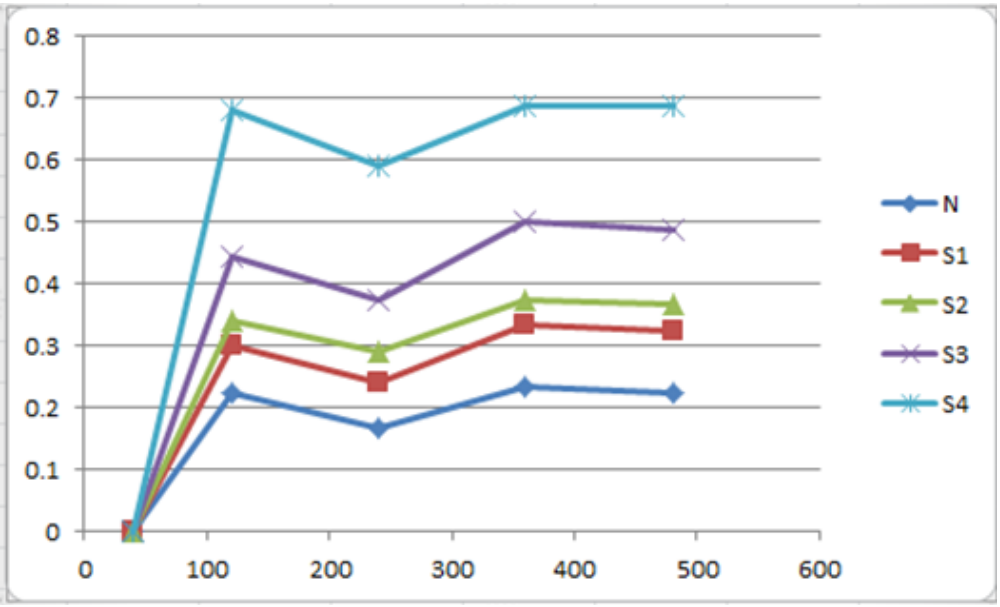


Fig 14: Water absorption study (graph) of silk/chitosan coated knitted silk scaffold

CHAPTER-6

CONCLUSION

6. CONCLUSION

Tissue engineering is often considered as an ultimately ideal medical treatment. To regenerate new tissues, this biomedical engineering utilizes three basic tools; cell, scaffold and growth factor. Tissue engineering can be applied in various disciplines, some of which are in case of bone and cartilage defects, muscular disorders, vascular defects etc. The most important role of tissue engineering is to rectify injury. Due to immune rejection and the inability of ligament to interact to the bone leads to failure of the organ. Hence the researchers are working to solve this problem. The current study had focused on fabrication of a knitted silk based scaffold for fibrocartilage regeneration. Coatings of biopolymer such as silk and chitosan were applied on the knitted silk scaffold for the growth of fibrocartilage. Lyophilization process was used for the coating of these polymers. Various characterisation studies such as FE-SEM, EDX, FTIR , XRD and water absorption proved that the biopolymer coated knitted silk scaffold is a novel material for the growth and proliferation of cells.

CHAPTER-7

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7.REFERENCES:

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